



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: M. Toland Attorney Docket No.: WEYE121907/22822C
Application No.: 09/700,037 Group Art Unit: 1651
Filed: July 2, 2001 Examiner: L.B. Lankford Jr.
Title: METHODS FOR CLASSIFICATION OF SOMATIC EMBRYOS

DECLARATION UNDER 37 C.F.R. § 1.132

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TO THE COMMISSIONER FOR PATENTS:

I, Mitchell R. Toland, do hereby declare that:

1. I am the inventor of the invention claimed in the above-identified patent application.
2. I had been working towards devising methods for automatically classifying somatic embryos according to their putative quantifiable characteristics, such as their tendency to successfully germinate, to tolerate adverse weathers, diseases, etc. My effort resulted in the methods as claimed in the above-identified patent application.
3. Specifically, I have discovered that, by applying a Lorenz curve classification algorithm to the raw image data collected from plant embryos of known quantifiable characteristics, I could develop a single metric classification model that can then be used to classify plant embryos of unknown quantifiable characteristics, according to their putative quantifiable characteristics.
4. According to one embodiment, the method of the present invention involves generally three steps. First, image data are obtained from reference plant embryos having known quantifiable characteristics. In a simple case, the reference plant embryos are divided into two

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sets: those of relatively high quality and those of relatively low quality based, for example, on a follow-up study.

Second, a metric value is calculated from the acquired raw digital image data of each embryo of known quality. These metric values are divided into two sets of metric values according to their known embryo quality. A Lorenz curve classification algorithm is applied to the two sets of metrics and a point on the Lorenz curve is used as a threshold value to arrive at a single metric classification algorithm. In one example described in the specification of the present application, at page 21, line 3 through line 16, a digital imaging set up for collecting digital images of a set of reference plant embryos of known quality is described. This set up was used in Example 4 at page 24, line 9. Example 4, beginning at page 26, line 5 describes that a classification method based on Lorenz curve classification algorithm was carried out on Douglas-fir genotypes 6 and 7. This classification method based on a Lorenz curve classification algorithm is described in more detail at page 27, line 17 through page 32, line 1 of Example 4. Page 19, line 1 through line 29, describes how a single metric value is correlated to classes of plant embryos of differing quality using a Lorenz curve classification algorithm.

The first row of Tables 4 and 5 lists the morphology classification and germination classification results from a single metric classification method developed from raw digital image data of embryos of known quality and a Lorenz curve classification algorithm. In the first entry of Tables 4 and 5, the metric used is the skewness coefficient (β_1) of all the intensity pixel values from the embryo end view. As described at page 16, line 18 of the specification, a coefficient of skewness is an exemplary metric. As described at page 18, line 24 through line 28, Lorenz curves are calculated for each metric in a set of metrics. The metric values of the extreme points of the Lorenz curves are used as the threshold values to make single metric classifications of the embryos. Values of a metric less than its threshold value are assigned to

one embryo quality class, and values greater than the threshold are assigned to the other embryo quality class.

In Example 4, the coefficient of skewness (β_1) is correlated with plant embryos classified based on morphological similarity and to normal zygotic embryos or germination classes, and this correlation was used to develop a single metric classification model using Lorenz curves as described above.

Once the single metric classification model is developed, image data is obtained of plant embryos of unknown embryo quality. The developed single metric classification model is applied to the raw digital image data from the plant embryos of unknown quality, and the quality of the plant embryos of unknown quality is classified.

5. As the above example illustrates, I was able to establish a clear correlation between raw image data collected from plant embryos and the plant embryos' quantifiable characteristics, by applying a Lorenz curve classification algorithm to the raw image data. I have demonstrated, in various examples shown in the specification, that based on such correlation, a single metric classification model can be developed and applied to embryos of unknown quality to classify them according to their putative quantifiable characteristics. I have successfully implemented some exemplary classification models, as shown in the specification.

6. I acknowledge that willful false statements, and the like, are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing therefrom. All statements made of my own knowledge are true and all statements made on information and belief are believed to be true.

18 Feb 2005
Date

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